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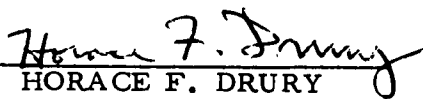
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ABSTRACT

This review of the control of circulation during hibernation begins with a general description of hibernation as it occurs in various groups of mammals and outlines the difficulties in the physiological study of hibernation. There follows a more detailed description of the three phases of hibernation -- entering hibernation, in deep hibernation, and waking from the hibernating state. The bulk of the review concerns research on rodents, which have been the most intensively studied of the animals which hibernate. It is emphasized that hibernation, at least in this group, is a precisely controlled series of physiological changes in which the animal maintains a homeostatic condition at all times in spite of profound changes in body temperature.

PUBLICATION REVIEW



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CIRCULATION IN MAMMALIAN HIBERNATION

SECTION 1. INTRODUCTION

Scattered among the orders of the Class Mammalia are various species which are considered to be "hibernators." The exact definition of "hibernation" is open to considerable argument, for it is a physiological spectrum, from the black bear (Ursus americanus) which remains inactive most of the winter but lowers its body temperature only 3° or 4° C (Hock, 1960) to the smaller bats of the Northern Hemisphere which remain virtually motionless for more than a month at a time with a body temperature near 0° C. The bulk of research in this field has been confined to the mammals which allow their body temperature to decline to a few degrees above the freezing point of water, and we have coined the phrase "deep hibernation" for this condition (Lyman, 1948). Deep hibernation certainly occurs among some of the Insectivora, Primates (Mouse lemur, fat-tailed lemur), Chiroptera and Rodentia. Most of the research on the subject of deep hibernation has been carried out on the vespertilionid bats, the Insectivore Erinaceus, and various species of rodents, including the Gliridae (dormice), the Marmotini (ground squirrels, woodchucks and chipmunks) and the hamsters Cricetus and Mesocricetus.

Because hibernation may differ, at least in detail, even from species to species, a list of the scientific and common names of the hibernators mentioned in this report is given below.

Order: Insectivora

<u>Erinaceus europaeus</u>	European hedgehog
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Order: Primates

<u>Microcebus</u>	Dwarf lemur
<u>Cheirogaleus</u>	Fat-tailed lemur

Order: Chiroptera

<u>Myotis lucifugus</u>	Little brown bat
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Order: Rodentia

<u>Mesocricetus auratus</u>	Golden hamster
<u>Sicista betulina</u>	European birchmouse
<u>Glis glis</u>	European dormouse
<u>Perognathus longimembris</u>	Little pocket mouse

Tribe: Marmotini

<u>Marmota monax</u>	American woodchuck
<u>Marmota marmota</u>	European marmot
<u>Citellus beecheyi</u> *	California ground squirrel
<u>Citellus franklini</u>	Franklin ground squirrel
<u>Citellus lateralis</u>	Golden-mantled ground squirrel
<u>Citellus parryi</u>	Arctic ground squirrel
<u>Citellus tridecemlineatus</u>	Thirteen-lined ground squirrel

* The nomenclature of this genus has been confused by the pronouncement (HersHKovitz, 1949) that Citellus should be called Spermophilus. Common usage of over half a century dictates the continued usage of Citellus.

Deep hibernation is characterized by a periodic abandonment of the warm-blooded state, accompanied by a profound lowering of the metabolic rate. All healthy hibernators may arouse from hibernation from time to time, using only heat from endogenous sources, and it seems to be typical of hibernation that periodic arousals take place throughout the season of hibernation, though the cause of these arousals is unknown (Kayser, 1960).

Hibernation in all of its phases contrasts sharply with enforced hypothermia. In the former the animal cools with little metabolic resistance; in the latter the thermogenic ability must be shattered before profound cooling can take place. During the whole period of hibernation the animal remains in control of its internal environment, and sometimes may be aroused by as light a stimulus as a touch. In hypothermia a process of deterioration takes place which eventually ends in death, and the animal cannot return to the warm-blooded state without being warmed artificially. In arousal the hibernator is capable of mobilizing its heat conserving and generating mechanisms so that the animal warms in a minimum of time in spite of a cold environmental temperature. The hypothermed animal shows vestiges of these same capabilities (Adolph and Richmond, 1955), but lacks both the endogenous ability to start the warming process and the physiological coordination necessary to complete it.

A part of the hibernator's success is undoubtedly due to the ability of the tissues and organs to function at low temperatures. In studies of peripheral nerve (Chatfield et al, 1948), heart (Lyman and Blinks 1959; Michael and Menaker, In manuscript; Tait, 1922), and nerve-muscle (South, 1961; Tait, 1922), it is clear that the specific tissue or organ in mammals which hibernate continues to perform usefully at lower temperatures than the counterpart from a mammal which cannot hibernate. Some evidence is accumulating that the tissues and organs of animals which hibernate undergo further

adaptation when the animals are exposed to cold (Kehl and Morrison, 1960; South, 1961), but the evidence is not as compelling as the data on the innate differences between hibernators and nonhibernators.

However, the ability to function at low temperatures will not by itself explain the phenomenon of hibernation. Hibernators can be forcibly hypothermed using the same techniques as used in nonhibernating mammals. In this case the potential hibernator will live for a longer time at a lower temperature than the hypothermed species which is incapable of hibernation. However, the hypothermed hibernator is incapable of arousal without heat from an outside source, and it will die in hypothermia within hours. On the other hand the hibernator will remain in natural hibernation for days, while retaining the ability to arouse at any time during the period.

The difference between the induction of hibernation and of hypothermia is one of the major unsolved problems in the physiology of hibernation. Many animals, notably some species of ground squirrels (Pengelley and Fisher, 1961), are very seasonal in their cycle. During the spring and summer they cannot be made to hibernate when exposed to cold and maintain as regular a body temperature as the common nonhibernating laboratory animals of the same size. During the autumn they usually hibernate at once when exposed to low environmental temperatures and allow their temperature to drop markedly even at laboratory temperatures of 23° to 25° C. The Mohave ground squirrel perhaps represents the extreme of this condition. In the desert it disappears in August and does not reappear until March. At usual laboratory temperatures it starts to "aestivate" in August, and its body temperature and metabolic rate are markedly reduced. Aestivation and hibernation seem to be physiologically identical except that the environmental and body temperature are not as low in the former as in the latter (Bartholomew and Hudson, 1960). Thus, this ground squirrel remains ready to aestivate or hibernate for more than half of the year. Other animals such as the golden hamster show no seasonal proclivity for hibernation but enter the hibernating state after an indefinite prolonged exposure to cold.

Certainly some sort of physiological preparation is involved in any of these animals. Seasonally hibernating ground squirrels and woodchucks fatten markedly prior to hibernation and may more than double their weight in the autumn months. Hamsters change the saturation of their depot fat when exposed to cold (Fawcett and Lyman, 1954) and tend not to hibernate when denied the ability to store food (Lyman, 1954). In many species which hibernate, a polyglandular involution of the endocrines during the autumn has been observed, but its causal relationship to hibernation has not been proved and hibernation has not been induced by abolition of any of the endocrine organs (Popovic, 1960a). Indeed, attempts to induce hibernation in animals which are not "ready" to hibernate have been signally unsuccessful. Fat ground squirrels may show a tendency to hibernate which is statistically significant

when compared to thin ones, but some of both groups will hibernate. Hamsters with saturated depot fat hibernate as readily as those with unsaturated fat. When changes such as low blood sugar or high serum magnesium, which have been observed in the hibernating mammal, are artificially produced in the awake animal, a torpor and lack of temperature regulation may result, but the animal is incapable of arousal without artificial assistance (Soumalainen, 1939).

There is another group of small hibernators which seems to require no preparation to enter the hibernating state. The European birchmouse (Sicista betulina) undergoes daily decline in body temperature which is indistinguishable from hibernation (Johansen and Krog, 1959) and the little pocket mouse (Perognathus longimembris) enters hibernation whenever sustenance becomes scarce (Bartholomew and Cade, 1957). Probably the vespertilionid bats belong to this group of hibernators also, for they lower their temperature when inactive in the cold at all seasons of the year, though the little brown bat, at least, may be incapable of natural arousal from 3° C during the summer season (Menaker, 1962).

Though preparation may play an important part in setting the stage for the hibernating state in many animals, the prepared hibernator cannot be forced into hibernation simply by exposure to extreme cold. The reaction to this stress is the same whether the animal is prepared or unprepared and results in an increase in its physiologic defenses against cold. If the cold is overpowering, the animal succumbs to hypothermia. Onset of hibernation, however, is a regulated and relatively passive occurrence which is not known to take place at an environmental temperature below 0° C and which may well transpire when the animal is in normal sleep. Not unnaturally, the changes which take place in the circulation during this time, and during the whole of the hibernating cycle, are of great importance. An understanding of the circulatory regulation may well supply many of the clues to the induction, maintenance, and arousal from hibernation, and for this reason considerable emphasis has been placed over the years on the circulatory physiology of mammals during hibernation.

The student of hibernation is limited in the techniques at his disposal by the peculiarities of hibernation itself. Because the onset of hibernation is unpredictable and, in our present state of knowledge, cannot be induced, chronic methods of recording are a necessity when studying the entrance into hibernation or the hibernating state itself. Furthermore, no potential hibernator will enter into the dormant state if it is physically restrained or rendered uncomfortable by the attachment of cumbersome recording apparatus. When the animal is in deep hibernation it cannot be moved or manipulated without running the risk of starting the process of arousal -- a process which involves a relatively rapid sequence of physiological changes which differ markedly from hibernation itself.

Given these limitations, it is natural that electrocardiographic studies, using unobtrusive indwelling electrodes, were used rather early in the modern studies of hibernation. Studies of the blood were also made, for samples could be obtained acutely with cardiac punctures which were rapid enough to be sure that no change had taken place during the withdrawal of blood. More recently the use of chronically implanted aortic and venous cannulae have shed some light on the various changes which take place during the hibernating cycle. Using pressure transducers, long-term records can be obtained as the animal enters hibernation, remains in hibernation and arouses from the hibernating state. Cardiac output can be estimated from A-V differences obtained from aortic and venous cannulae or by dye dilution techniques. Drugs may be injected through the cannulae with the assurance that the effect observed is due to the drug alone and not to the physical disturbance of the injection itself. Though many of the common techniques for studying blood flow are denied the student of hibernation, still the fact that large variations of body temperature occur during hibernation has a particular advantage, for differences in temperature in various parts of the body give presumptive evidence of restriction of blood flow.

By using these methods, reliable data have been obtained concerning the physiological changes which occur during hibernation. As is to be expected, the changes in the circulation are an integral part of the overall changes, and a study and discussion of one cannot be undertaken without considering the others. Caution must also be observed in drawing overly broad conclusions from observations on one species. The evidence is persuasive that hibernation is polygenetic in origin (Lyman and Blinks, 1959), and though it may be hoped that the same general principles apply in the whole mammalian class, still it is quite clear that there are large variations in detail. For this reason the species will be identified whenever it seems appropriate in this report.

SECTION 2. SUMMARY

This review of the control of circulation during hibernation begins with a general description of hibernation as it occurs in various groups of mammals and outlines the difficulties in the physiological study of hibernation. There follows a more detailed description of the three phases of hibernation — entering hibernation, in deep hibernation, and waking from the hibernating state. The bulk of the review concerns research on rodents, which have been the most intensively studied of the animals which hibernate. It is emphasized that hibernation, at least in this group, is a precisely controlled series of physiological changes in which the animal maintains a homeostatic condition at all times in spite of profound changes in body temperature.

When the animal enters hibernation, the heart rate slows before body temperature declines. The slowing is accomplished by skipped beats and by slowing of even beats. The skipped beats become prolonged as the body temperature declines. They can be abolished by parasympatholytics and hence are parasympathetically induced. Peripheral resistance increases as hibernation deepens, and serves to keep blood pressure reasonably high in spite of a very slow heart rate. Peripheral resistance can be reduced by adrenergic blocking agents and by acetylcholine and hence is considered to be sympathetically controlled.

In deep hibernation, with heart rates of 2 - 9/minute, the heart rate cannot be slowed either by stimulation via chronically implanted vagal electrodes or by infusion of parasympathomimetics. Only after the heart rate has accelerated, and remained at the accelerated rate for some minutes, is it possible to slow the heart by vagal stimulation or by parasympathomimetics. This change in threshold is dependent on heart rate and time rather than temperature. Infusion of parasympatholytics causes little or no acceleration of heart rate. Thus, it is postulated that the heart rate is not influenced by the parasympathetic system during deep hibernation, and it is further postulated that variations in rate are reflections of sympathetic tone.

Infusion of parasympathomimetics invariably causes cardioacceleration and often arousal from deep hibernation. Though in some cases the cardioacceleration can be regarded as compensatory to a drop in blood pressure, in other cases acceleration occurs at once, with no blood pressure change. The acceleration is blocked by sympatholytics, by ganglionic blockade and by curare. Infusion of the parasympathetomimetics always produces a long lasting burst of muscle action potentials followed by cardioacceleration, and physically disturbing the animal produces the identical result. It is postulated that the burst of muscle action potentials reflexly increases the heart rate via the cardioaccelerator fibers of the sympathetic system, and this is the basic sequence involved in arousal.

During arousal, the heart rate increases before an increase in body temperature. Vasoconstriction of the posterior and periphery causes the anterior part of the body to warm faster than the posterior. Heart rate increases rapidly but cannot be made to beat faster at any given temperature by infusion of norepinephrine or atropine; hence, it is being driven as fast as possible by the sympathetic system and not slowed by the parasympathetic. Vasoconstriction of the posterior can be abolished by pretreatment of the hibernator with a sympatholytic, or by acute treatment with acetylcholine. Thus, the vasoconstriction is sympathetically mediated. In the last stages of arousal, when the anterior temperature has reached 37° C and the posterior temperature is rising rapidly, vasoconstriction of the posterior can be briefly reimposed by norepinephrine infusion. Entering hibernation contrasts with arousal. In the former, the function of the circulation is chiefly

the carrying of nutrients and removal of metabolites. Arousal is metabolically expensive, and the circulatory modifications during this time hasten the process by differential vasoconstriction and by contribution of heat from the rapidly beating heart.

SECTION 3. THE HIBERNATING CYCLE

Mammals which hibernate can be regarded conveniently as existing in one of four states. (1) They may be "active," with the usual thermoregulatory responses common to most mammals and a body temperature ranging within two or three degrees of 37° C. (The word "active" is semantically inadequate for this state because it includes both the awake and the sleeping condition, but it is used here with this reservation.) (2) The animal may be entering hibernation. (3) Entrance into hibernation passes into hibernation itself with no clear physiological demarcation. (4) Finally, the animal may "arouse" from hibernation, during which time it is in a changing physiological state which clearly differs from the three other conditions. Each of the four states are discussed below.

Active

Hibernators in the active condition do not differ markedly from nonhibernators. In all but the very small species the homeothermism of mammals which hibernate is much the same as in nonhibernating mammals of the same size. At least some of the hibernators, such as the European ground squirrel (Popovic, 1951), are capable of remarkably high maximal metabolism if exposed to the thermal stress of cold. No comparative studies have been made on the condition of the circulation in the active hibernator and the nonhibernator, but it is reasonable to assume that vasoconstriction and dilation form just as important a part of the temperature regulatory mechanisms in the former as in the latter.

Recent studies on two very small terrestrial mammals which hibernate show that they differ from nonhibernating mammals of the same size because they tend to lose their thermoregulatory ability when exposed to cold. The birchmouse (weight 7 to 16 grams) has a marked diurnal temperature fluctuation, with the body temperature dropping close to the temperature of the laboratory during the early morning hours and rising to 37° C in the afternoon and evening. If exposed to temperatures below 15° C, this species compensates with an increased oxygen consumption for a variable period of time and then enters a condition which is indistinguishable from deep hibernation (Johansen and Krog, 1959). The little pocket mouse (weight 6.5 to 10 grams) enters hibernation whenever it lacks food, even at an environmental temperature of 20° C (Bartholomew and Cade, 1957).

Many bats of the northern hemisphere are known to lower their body temperature whenever they become inactive. If a small mammal is at a disadvantage in the cold because of its large surface to mass ratio, the bats must face an even greater problem with their highly vascular wings. When actively flying, the wing membranes may become engorged with blood and thus act as dissipators of heat. When inactive, little blood flows to the wings and the wings themselves may give some protection against the cold (Reeder and Cowles, 1951).

Entrance

The exact point at which an animal enters hibernation is difficult to define. Many hibernators undergo small undulations of body temperature and heart and metabolic rate before starting the real decline into deep torpor. The California ground squirrel allows its body temperature to drop to a lower level each night but arouses from hibernation daily, until finally the state of deep hibernation is attained (Strumwasser, 1960). These graded entrances and exits from hibernation are rather precisely timed, but once deep hibernation has been reached the periods in hibernation are of longer duration and are less exact. We have not observed this clocklike type of entrance into hibernation in either the golden mantled or thirteen-lined ground squirrel. As the body temperatures of the woodchuck (Lyman, 1958), thirteen-lined ground squirrel (Lyman and O'Brien, 1960), and California ground squirrel (Strumwasser, 1960) decline, the animals sometimes undergo periodic bouts of shivering which can be sufficiently violent to stop the downward course of the body temperature or even cause a brief rise. As measured in the thirteen-lined ground squirrel, an increase in blood pressure and rise in heart rate always accompany the shivering. These steplike entrances into hibernation are usually manifest only at the beginning of the hibernating season and do not occur at all in other species such as the golden hamster (Lyman, 1948) or the pocket mouse (Bartholomew and Cade, 1957).

In all species observed to date, there is a marked decrease in heart rate before any recordable change in body temperature as the animal enters hibernation. Landau (1956) has shown that respiratory rate slows even before heart rate in the ground squirrel, and a decrease in metabolic rate before the drop in body temperature has been reported in the woodchuck (Lyman, 1958). These decreases in vital functions may be simply manifestations of normal sleep, but in our present state of knowledge we cannot say when sleep gives way to hibernation.

The general picture of the onset of hibernation is a passive abandonment of the usual thermogenic armamentarium. Actually, many species often manifest a period of hyperactivity just prior to hibernation, but the body

temperature still does not decline before the other vital functions (Figure 1). The pocket mouse is perhaps an exception to this, for brief periods of violent activity occur as the body temperature is dropping. The periods of activity cause no change in the constant decrease of the temperature of a thermocouple implanted in the lumbar muscles (Bartholomew and Cade, 1957). Unfortunately, the state of the circulation during this time has not been examined, and we are also ignorant of the magnitude of change in the metabolic rate.

The slowing of the heart as hibernation begins in the thirteen-lined ground squirrel is accomplished both by a prolongation of the time between individual beats and by periodic skipping of beats (Figures 2a, 2b). As hibernation deepens the skipped beats develop into prolonged periods of asystole, which occur quite regularly and are followed by a series of faster, regularly spaced beats which bring the blood pressure to a higher level (Figures 3a, 3b). The prolonged asystole is often associated with apnea, and a deep inhalation is apt to precede the first of the even beats.

If heart rate and heart temperature of C. tridecemlineatus are plotted against time, the result, unless the animal undergoes brief periods of shivering and cardioacceleration, is two smooth curves, with the heart rate reaching its minimum value in about nine hours while the body temperature is still declining. A plot of the heart rate counting only the even beats results, of course, in a faster rate at any given time or temperature. More important, however, is the fact that the heart rate curve in this latter case is more uneven, for the relation of rate to time or temperature is less precise. Thus, the skipped beats and asystoles serve to maintain a more exact correlation of rate with temperature and time in this species. In other rodents such as the woodchuck (Lyman, 1958) and the California ground squirrel (Strumwasser, 1960) the variation in rate with temperature is much less exact.

Parasympathetic action undoubtedly induces the skipped beats and asystoles, as well as contributing to the slowing of the even beats, for in the thirteen-lined ground squirrel atropinization can abolish the asystoles and cause the even beats to occur more rapidly (Figures 4a, 4b). An animal so treated will still approach the hibernating state, for the body temperature will continue to decline though the heart rate is much higher than normal at any given body temperature. In this situation typical deep hibernation is not attained even when the body temperature ceases to decline and remains unchanged for hours, for the heart rate is more rapid than is ever observed in the untreated animal.

Thus, parasympathetic activity hastens and modulates the decline in heart rate as the animal enters hibernation. Even when the heart rate has reached the level seen in deep hibernation (i. e. below ten beats a minute)

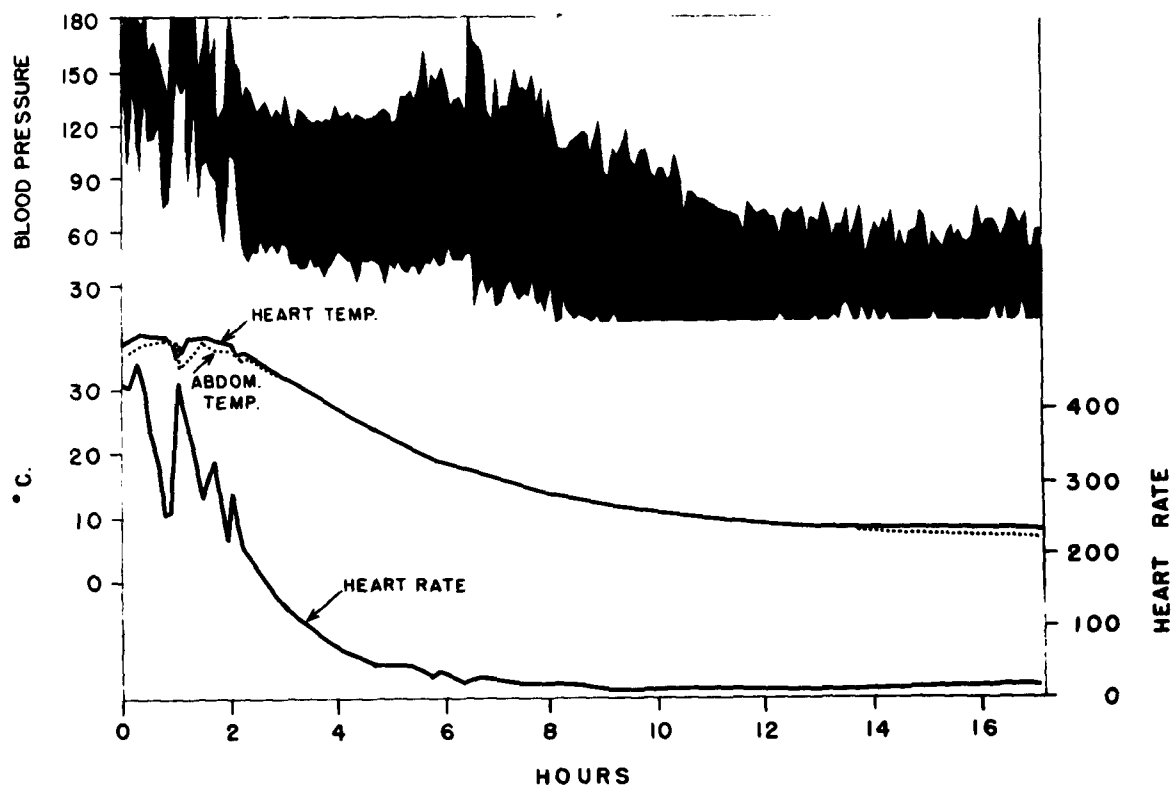


FIGURE 1

Thirteen-lined ground squirrel entering hibernation. Drop in blood pressure and heart rate always precede decline in body temperature. Blood pressure in dark area is highest systole and lowest diastole recorded every four minutes for a one-minute period.

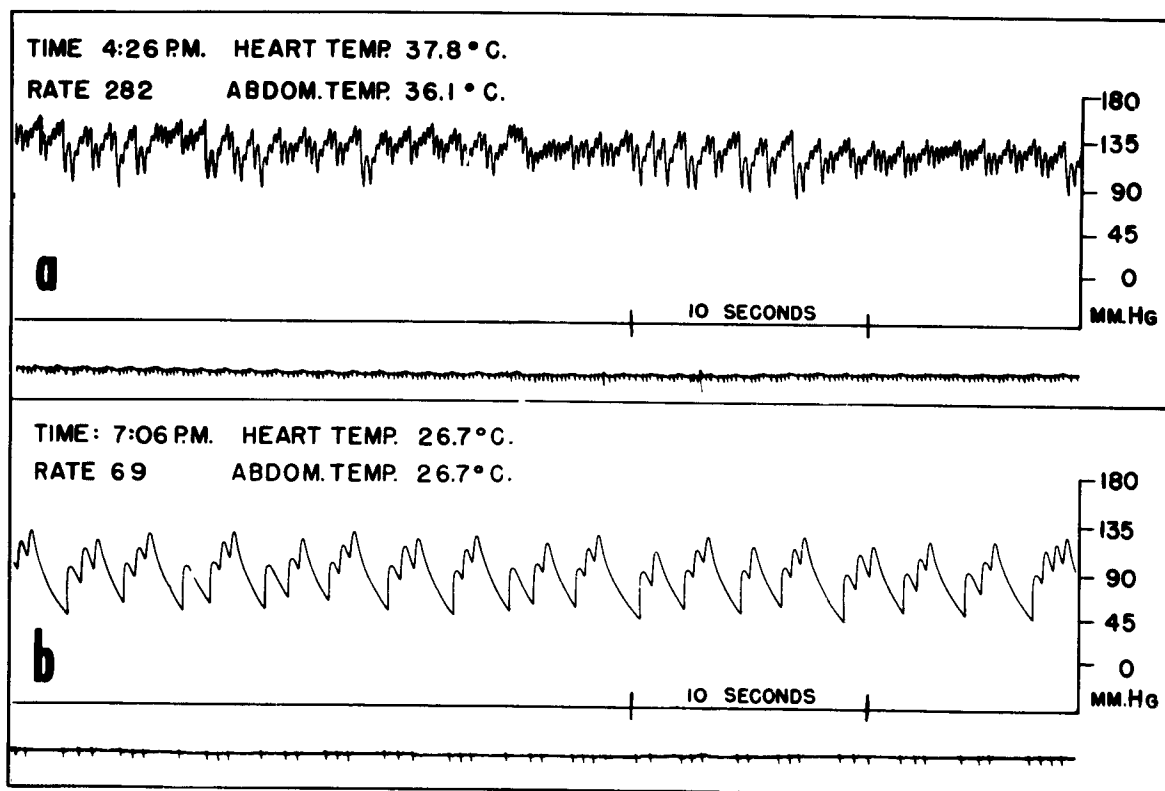


FIGURE 2a

Blood pressure and EKG of same animal as in Figure 1 entering hibernation. Skipped beats appear before drop in heart temperature. 4:26 p.m. = 1.5 hours in Figure 1.

FIGURE 2b

Same animal. Note even pattern of beats and asystoles.

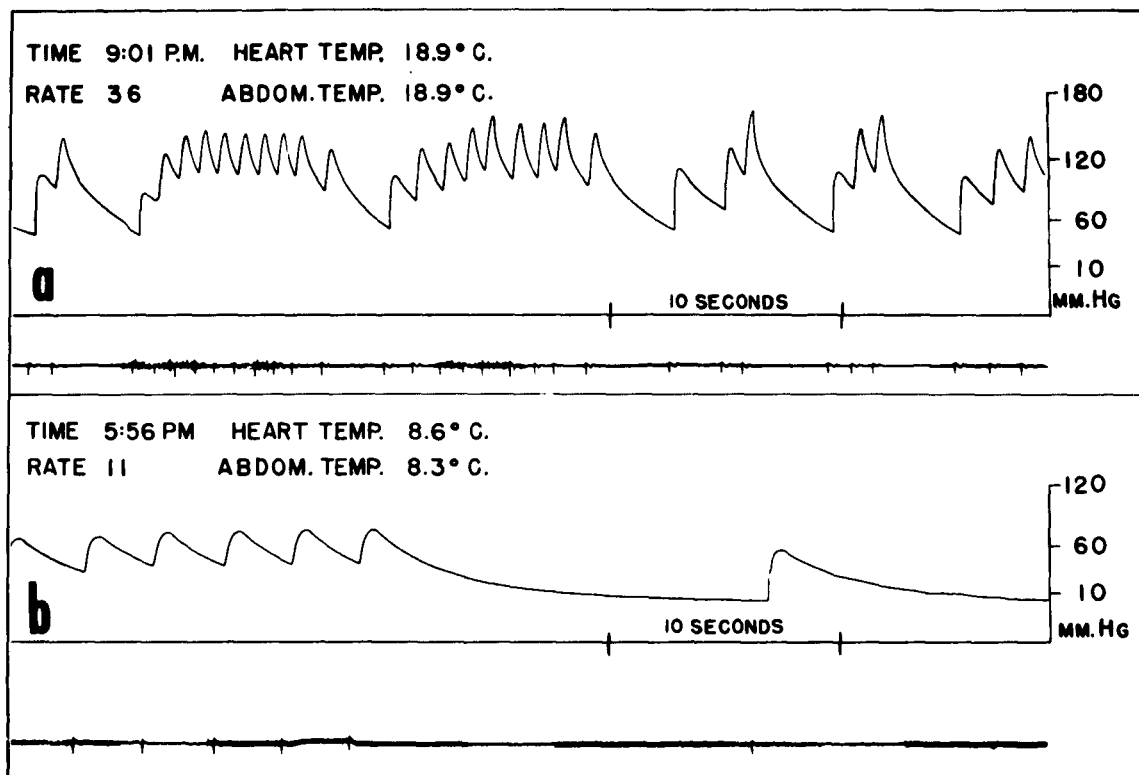


FIGURE 3a

Same animal as Figure 2, showing transient increase of heart rate and appearance of muscle action potentials on EKG.

FIGURE 3b

Same animal in deep hibernation. Blood pressure tube slightly plugged. Blurring of EKG is 60 cycle artefact.

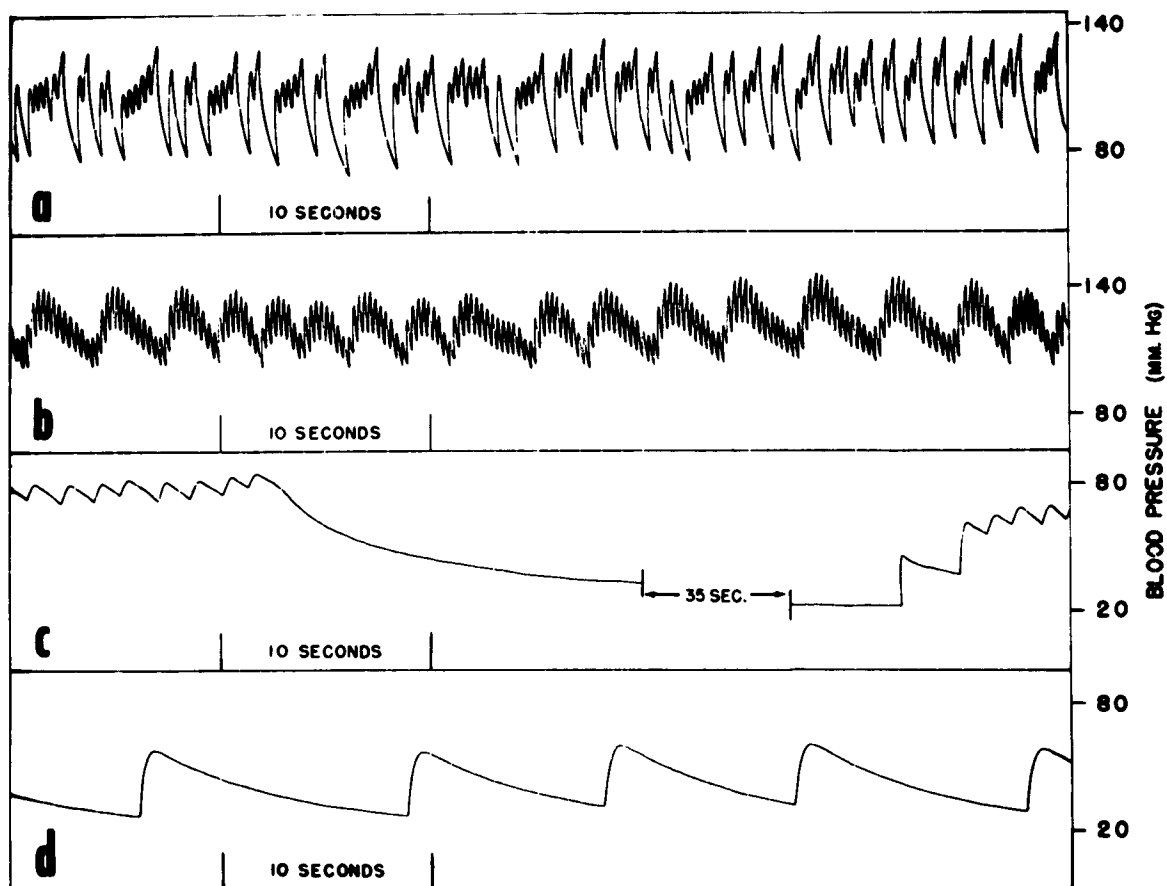


FIGURE 4a

Blood pressure of untreated ground squirrel entering hibernation. Heart temperature = 29.5° C.

FIGURE 4b

Same animal 30 seconds after a small amount of atropine (1.37 mg/kg). Heart temperature = 29° C.

FIGURE 4c

Atropinized animal (18.5 mg/kg) entering hibernation showing prolonged asystole. Heart temperature = 14.5° C.

FIGURE 4d

Same atropinized animal in hibernation showing pulse pressure typical of normal hibernation. Heart temperature = 10.5° C.

atropinization will cause a speeding of the heart if the body temperature is still declining. However, atropine has little or no effect once the animal is in deep hibernation with an unchanging body temperature about 2° C above the environmental temperature. Under such conditions the rate may become slightly more even after atropinization, but the increase in rate never amounts to more than one or two beats per minute.

Although it is not always the case, asystoles can occur in the atropinized animal entering hibernation but their pattern and timing is different than in the untreated ground squirrel. In these cases a period of asystole occurs after many minutes of evenly spaced, rapid heart beats, and this period is greatly attenuated (Figure 4c), in one case lasting 80 seconds. During this time the respiratory rate becomes increasingly rapid, as if the animal were in respiratory distress, and the heart rate, when beating starts again, is faster than it was before the asystole occurred. We have suggested that this type of asystole occurs when sympathetic influence is withdrawn and the heart, which has been beating at an abnormally high rate for its temperature, loses its inherent rhythmicity (Lyman and O'Brien, In manuscript). Alternatively, the high rate at low heart temperature may use up some essential substance faster than it is produced and the heart may stop until the substance accumulates. A similar situation would occur if some inhibiting by-product accumulated faster than it could be removed. An interesting parallel is found in the periodic cessations of heart beat in the denervated cat heart under influence of veratramine (Kosterlitz et al, 1955).

The slowing of the heart during entrance into hibernation which is not due to parasympathetic action might be due to temperature alone, but this in no way explains why the body temperature declines as the animal enters hibernation. If the prolonged asystoles which may occur in the atropinized animal are due to a letup in sympathetic activity, then it is reasonable to assume that sympathetic activity is reduced, at least from time to time, in the untreated animal as well. Certainly the cardioaccelerating action of the sympathetic system is in abeyance during deep hibernation, for during this period the heart can easily be accelerated by a variety of drugs, including sympathomimetics, as will be detailed below. The behavior of the atropinized animals with prolonged asystoles lends credence to the theory that the sympathetic effect lessens as deep hibernation approaches. Under these conditions the periods of cardioacceleration may become progressively shorter as the body temperature declines, and the periods of asystole may occur more and more evenly. Finally, when the body temperature runs level within 2° C above the environmental temperature, the periods of single heart beats are so even that they appear identical to the untreated animal in deep hibernation (Figure 4d). In such a case, it seems probable that the animal reached true deep hibernation without benefit of parasympathetic action because the sympathetic tone was gradually reduced. In atropinized animals without prolonged asystoles, on the other hand,

sympathetic tone may be unusually high, thus preventing the ground squirrel from attaining typical hibernation.

Some of the earlier investigators believed that the heart rate during hibernation was temperature-dependent and attempted to correlate heart rate with van't Hoff's rule (Barcroft, 1934; Endres et al, 1930). In these correlations no clear distinction was made between the heart rate during entrance into and arousal from hibernation. We now know that the two rates are vastly different at the same temperature. Furthermore, the same animal may have a different temperature-rate curve during two separate entrances into hibernation (Figure 5). This is not to say that the heart rate is not temperature-dependent in part, but only to emphasize that there can be a wide variation of rate depending on the other factors which are influencing the heart at the time.

Dawe and Morrison (1955), studying two species of ground squirrels and the European hedgehog, were first to point out that the heart rate does not decline linearly with temperature as the animals enter hibernation. In the species studied they found a break in the curve at about 20° C, so that the hearts beat at much lower temperatures than would be predicted if the curve formed between 35° and 20° C were extrapolated to 0° C. Using isolated hearts, Lyman and Blinks (1959) showed that this break in the curve was typical of at least four rodent species which hibernate, and Michael and Menaker (In manuscript) have demonstrated the same response in the isolated heart of the little brown bat. Nonhibernating rodents closely related to the hibernators had a nearly linear relationship of heart rate to temperature, and the hearts ceased to beat at temperatures which were well above the typical temperatures of deep hibernation. The isolated heart of the world's most primitive living rodent, the mountain beaver (Aplodontia rufa), which does not hibernate, has a rate which declines linearly with temperature and ceases to beat at 10° C. This serves to emphasize that hibernation is not, as has often been claimed, a primitive condition.

Thus, the hearts of animals which hibernate each have a typical temperature-rate curve, all of which are characterized by a break from linearity and an ability to function efficiently at very low temperatures. It must be on this curve that the nervous and other influences play to produce the observed rate each time the animal enters hibernation.

The decline in heart rate with the onset of hibernation is accompanied by a marked decrease in systolic and diastolic pressure in the thirteen-lined ground squirrel (Figure 1). Though this change is striking, the blood pressure remains within the range observed in the normal conscious animal. As hibernation deepens the blood pressure decreases, the pulse pressure increases, and the diastolic run-off time becomes more attenuated (Figures 2 and 3). If observed from the same systolic pressure, the slope of

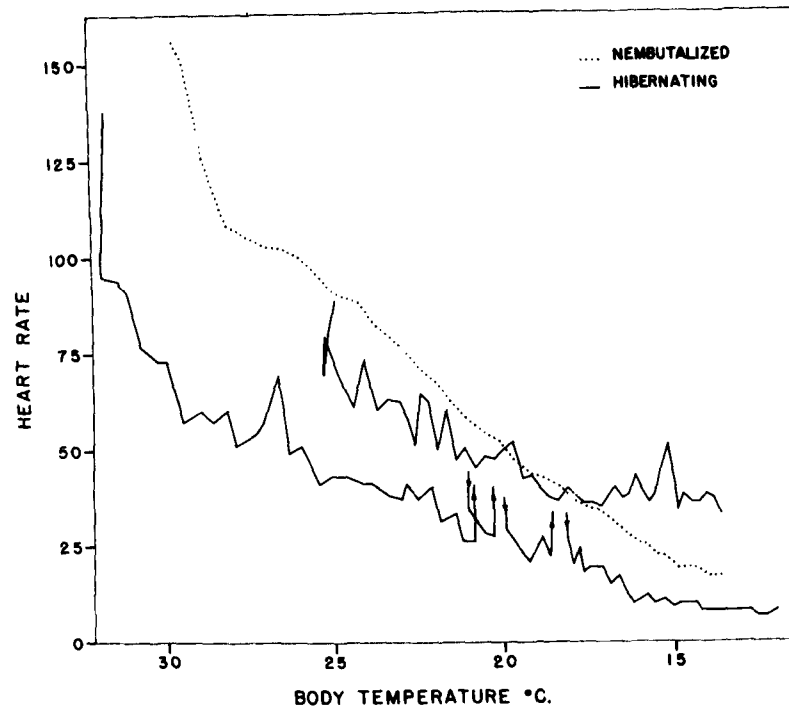


FIGURE 5

Heart rate of woodchuck during two successive entrances into hibernation, and under nembutal. Arrows indicate cardioacceleration and decline during bouts of shivering.

the angle which the diastolic run-off tracing makes with the perpendicular will give an estimate of the peripheral resistance. Plotted against body temperature, the increase in this angle is linear with the declining temperature. Thus, it appears that peripheral resistance increases with deepening hibernation and it is this increase which keeps the blood pressure within reasonable levels even with heart rates as low as three or four a minute.

Part of the increase in peripheral resistance is undoubtedly caused by the increased viscosity of the blood and other changes such as stiffening of the vascular walls which may be caused by cold; but a larger moiety must be caused by vascular tone, for if the ground squirrel is disturbed, or if a vasodilating drug such as acetylcholine is introduced via the aortic cannula, a dramatic shortening of run-off takes place at once without any change in body temperature. The maintenance of vascular tone and generalized mild vasoconstriction may well be one of the important differences between the hypothermed and the naturally hibernating animal. Popovic (1960b), working with rats and ground squirrels, has shown that animals which maintain their blood pressure during the first hours of hypothermia have a better prognosis for long survival. The heart rate in hypothermia is much faster at the same temperature than in an animal entering hibernation, and high blood pressure in the former must be maintained at the expense of a rapidly beating heart.

In the active thirteen-lined ground squirrel the temperature near the heart is higher than the abdominal temperature, but both temperatures are the same during most of the entrance into hibernation, though the abdomen becomes a bit cooler than the heart as hibernation deepens (Figure 1). This indicates an even blood flow throughout the body, with no specific areas of vasoconstriction. The same situation occurs in the woodchuck, though because of its large size and longer circulation time the differences in temperature from various parts of the body are somewhat magnified. It is probable that the blood of all hibernators is warmest at or near the heart and is cooled as it reaches the periphery.

Strumwasser (1960) has described the relationship between the temperature of the skin of the dorsum and that of the brain as the California ground squirrel enters hibernation. Using sensitive continuously recording skin-surface and deep brain thermistors, he reports that the decline in brain temperature actually consists of a series of alternate plateaus followed by relatively rapid temperature drops. According to his observations, a bout of shivering is followed by a plateau in brain temperature. Prior to the decline in brain temperature there is a rise in skin temperature. Thus he visualizes the mechanism of the "step-down" in temperature as a vasodilation and cooling of the blood in the exposed skin of the back which then is reflected in a cooling of the brain. During steep temperature drops there is a reduction of muscle tone, but actual shivering can occur during less precipitous declines. Using thermocouples located subcutaneously near the

heart, on the back and in the abdomen, we have recorded no such small, even, step-like declines of body temperature in thirteen-lined ground squirrels (Lyman and O'Brien, 1960) and woodchucks (Lyman, 1958), nor have we observed a correlation between subcutaneous back temperature and temperature near the heart. The method of recording may explain the difference. On the other hand, it may be that the California ground squirrel has developed a physiological refinement that is absent in its kindred species. "Step-downs" and plateaus in body temperature have not been reported in animals such as the pocket mouse, birchmouse and Mohave ground squirrel, which enter hibernation or aestivation with one precipitous decline in body temperature (Bartholomew and Cade, 1957; Bartholomew and Hudson, 1960; Johansen and Krog, 1959). The concept of the alternating vasodilation and vasoconstriction of a cool area as a means of controlling the reduction in temperature is attractive, however, for it could explain changes in blood pressure which have been observed as the animal enters hibernation (Lyman and O'Brien, In manuscript).

It would not be surprising that one type of hibernator might have different vascular control than another, for species differences are most apparent during entrance into hibernation and in the deeply hibernating state. This is particularly apparent when the electrical activity of the brain is considered. In the golden hamster, for example, the cerebral cortex is electrically inactive at any amplification when the brain temperature is below 18° to 20° C (Chatfield et al, 1951). This animal is totally inert as it enters deep hibernation. The European ground squirrel (Kayser et al, 1951) and the American woodchuck (Lyman and Chatfield, 1953) have some spontaneous cortical electrical activity at temperatures as low as 6° to 7° C. At this temperature both species are capable of spontaneous or induced uncoordinated movements. The birchmouse may have bursts of slow electrical waves which are often of fairly high voltage at an oral temperature of 2.5° C and will vocalize if disturbed at a rectal temperature of 4.5° C (Andersen et al, 1960).

In the California ground squirrel, well organized cortical waves persist to brain temperatures of 6.1° C or below, and the electrical activity of the motor cortex can be correlated with the periodic onset of shivering (Strumwasser, 1960). In this animal rather complex behavior patterns such as cocking the ears or vocalization persist even in deep hibernation, so that a fairly clear-cut relationship of electrical activity of the cortex and general mobility while entering hibernation appears to exist among the animals studied to date. We do not know whether this parallelism can be applied to the control of the circulation as well. Presumably circulation would be governed at subcortical levels, but Strumwasser's observations suggest a positive correlation between cortical activity and circulatory control.

In Hibernation

Only an arbitrary line can be established between the final stage of entrance into hibernation and deep hibernation itself. It might be decided that deep hibernation begins when the body temperature becomes level a degree or two C above that of the environment. This definition is complicated by factors such as the variation in environmental temperature. An animal with a body temperature of 8° C at an environmental temperature of 7° C would be in deep hibernation, while with the same body temperature and an environmental temperature of 3° C it would be entering hibernation, even if the physiological states were indistinguishable. However, a fair generalization is that deep hibernation is basically a steady-state condition, while both entrance into and arousal from hibernation are characterized by an unsteady state or constant change.

It is because deep hibernation is a delicately balanced steady state that it is so difficult to study. A mechanical stimulation may or may not start the process of arousal, depending on the unpredictable condition of the animal. This limitation has often not been appreciated by readers who are not familiar with the field. For example, there are many reports in the literature involving the subcutaneous injection of drugs into hibernating animals, but one can never be sure whether the observed result was pharmacologically or mechanically initiated.

Most animals are curled in a tight ball in hibernation, with the hair of the dorsum piloerected, their head tucked beneath their tail, and the tail, if it is long and bushy, wrapped around the body. If the tail of the species is bare or partly naked it is very often curled beneath the body. Only the back (and tail in bushy-tailed species) appears above the nest, which is usually well formed of insulative materials. Occasionally in the laboratory a ground squirrel may hibernate in a supine position or a hamster may hibernate outside its nest, but this is a relative rarity. The hibernating position suggests that heat conservation is an important factor in hibernation, yet at a steady environmental temperature the warmest body temperature is rarely more than 2° to 3° C above the environment. As with the animal entering hibernation, the area near the heart is the warmest part of the body (Adolph and Richmond, 1955; Lyman, 1958) but other areas are less than a degree below this in temperature. The curled position tends to curtail the flow of blood to the abdomen (Lyman, 1958), but deep rectal temperature is but a fraction of a degree below that of the heart. Bats are the only mammals which are extended in position during hibernation and their temperature throughout the body is virtually the same as the environment.

In the California ground squirrel it has been reported (Strumwasser, 1959) that the skin of the dorsum remains two or more degrees colder than that of the brain (which is, presumably, slightly cooler than the heart).

At a steady environmental temperature the brain temperature remains unchanged, but the skin of the dorsum shows a slow rise of about 0.5°C and a faster fall which occurs in a wave lasting about 15 minutes. Superimposed upon the wave of declining temperature a series of small oscillations having an amplitude of 0.2°C and lasting about 2 1/2 minutes has been observed. In the dead animal as well there are even oscillations in skin temperature, but because these oscillations are of a different frequency and amplitude, Strumwasser (1959) believes that the waves observed in the living hibernator are largely physiological in origin and represent circulatory adjustments which help to maintain the deeper body temperatures at such a steady state. As with the conflicting reports on entering hibernation, evenly occurring fluctuations of subcutaneous temperatures have not been seen in Citellus tridecemlineatus or other hibernating genera in which suitable recordings have been made. Blood pressure changes do occur in hibernating C. tridecemlineatus which would indicate changes in the vascular bed. These changes, however, take place over a long period of time and occur unevenly — in no way resembling the even 15-minute wave reported in the surface thermistor of Citellus beecheyi.

The relationship of deep body temperature and environment obtains in all hibernators at steady ambient temperatures between 3° and 15°C . If the environmental temperature drops below 3°C , the metabolic rate increases along with the heart rate and the animal maintains a larger gradient between its own body temperature and its surroundings. Hibernation may continue for many hours with an increased metabolic rate, or the stimulus may be sufficient to start the arousal process. This residuum of temperature control has been reported for such widely separated groups as bats (Hock, 1951), dormice (Wyss, 1932) and hamsters (Lyman, 1948) and it doubtless exists in all hibernators. It is not infallible, however, for the animal cannot respond fast enough to rapid cooling of the environment and may die either because of failure of organ function due to cold or because of actual freezing of the tissues. The circulatory adjustments in response to freezing cold have not been studied and would provide a useful avenue of research.

The heart rate in hibernation at a steady environmental temperature varies greatly from day to day and may even vary from hour to hour. Thus, in a series of 21 hamsters with cheek pouch temperatures of $6^{\circ} \pm 1^{\circ}\text{C}$ we have reported heart rates between 4 and 21 beats per minute (Chatfield and Lyman, 1950), though the rates above 15 per minute in this series may represent animals in an unsteady state (Lyman, 1951). In the tribe Marmotini, rates above 10 beats per minute with body temperatures of 5° to 8°C are very rare. Even heart rates as low as three beats a minute have been observed in many hibernating species and uneven rates can be as slow as one beat a minute for short periods. Strumwasser (1959) finds that the heart rate of the California ground squirrel is slower and more

regular just as the animal approaches deep hibernation than it is at the steady temperature of the hibernating state. This has not been observed in the hedgehog, the Arctic ground squirrel or in Franklin's ground squirrel (Dawe and Morrison, 1955) and does not occur in the thirteen-lined ground squirrel (Lyman and O'Brien, 1960).

The spacing of the heart beats may take any one of several forms. Beats may be fairly evenly spaced, though never precisely even. Alternatively, a series of beats may occur followed by a long period with no beat, and the pattern may be repeated; the latter sequence is apt to occur when the animal has just entered hibernation. Dawe and Morrison (1955) have illustrated a heart beat pattern in Franklin's ground squirrel in which a series of rapid beats lasting two or three minutes is followed by a much slower rate for about seven minutes. A similar pattern occurs in the thirteen-lined ground squirrel, with the blood pressure rising during the rapid beats and the pulse pressure enlarging during the slow beats (Figure 3b). Usually there is a period of apnea during the prolonged diastole, and a deep inspiration takes place before the next systole, but this is not always the case.

The changes in blood pressure which have been observed in hibernating C. tridecemlineatus and C. lateralis often, but not invariably, reflect a change in heart rate, with the higher rate resulting in a higher blood pressure. In C. tridecemlineatus systolic pressures can vary between 40 and 90 mm. Hg and diastolic pressures between 7 and 40 mm. Hg. (Although virtually nothing is known about the activity of the kidney in deep hibernation, it may be assumed that the mean blood pressure is high enough to provide for some renal function.)

Occasionally, in animals entering into or in deep hibernation, complete sequences of myocardial depolarizations are recorded with little or no change in pulse pressure. These may occur at fairly evenly spaced intervals and sometimes take the form of extra systoles (Figure 6) (Dawe and Landau, 1960; Lyman and O'Brien, 1960). It is interesting that similar depolarizations have been observed in isolated chilled hearts (Lyman and Blinks, 1959) and that they also occur clinically during hypothermic surgery. Obviously this could lead to some confusion in counting the number of useful beats per minute if only the EKG is used.

The electrocardiograms of animals in hibernation show no outstanding peculiarities. Sarajas (1954), using the hedgehog, and Dawe and Morrison (1955), using this species and the Franklin and Arctic ground squirrels, found that the time interval between beats is the most important factor in slowing the heart rate, but the lengthening of the various intervals of the depolarization and recovery process also contributes an important fraction of the total slowing. The lengthening of the intervals in the P-T complex is disproportionately greater at the low temperature of hibernation, so that the

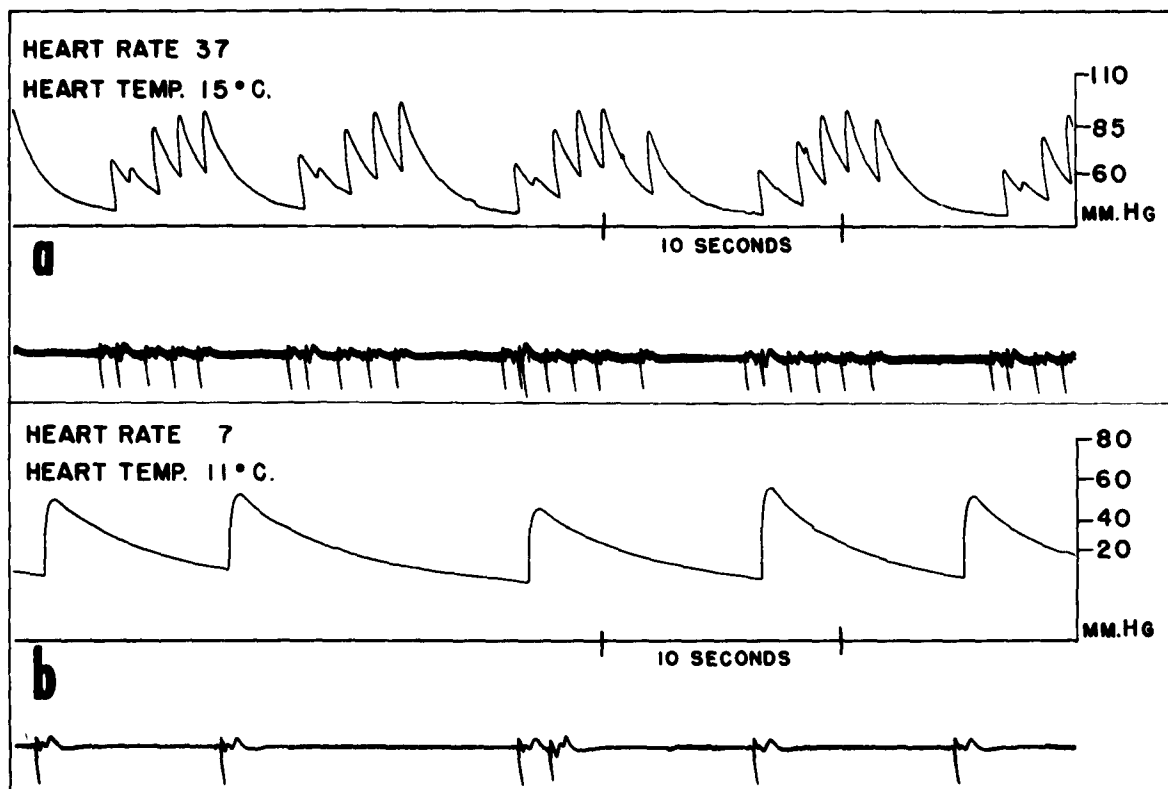


FIGURE 6a

Animal entering hibernation. Evenly occurring electrical depolarizations with little or no change in pulse pressure.

FIGURE 6b

Extra systole with no change in pulse pressure.

curves of plots of interval length against temperature break rather sharply at about the temperatures where the hearts of nonhibernators cease to beat altogether. Nardone (1955) interpreted a notched R wave in the electrocardiogram of C. tridecemlineatus as left bundle branch block. It is probable, however, that his animals were in the first stages of arousal rather than in deep hibernation as the rates he reported are high and the same type of notching can occur in the electrocardiogram of the hamster in the first stages of arousal (Chatfield and Lyman, 1950). Dawe and Morrison (1955) do not illustrate a notched QRS in either the Franklin or the Arctic ground squirrel nor was it found in the European marmot (Castellano et al, 1957), though it has been reported (Dawe and Landau, 1960) in C. tridecemlineatus which were decapitated during hibernation.

A-V dissociation in hibernation was first reported by Buchanan (1911) for the dormouse and bat and has been variously reported in other hibernators (Lyman and Chatfield, 1955). Many of the earlier results are open to question, however, because of the difficulty of recording the low voltage P wave. Furthermore, in many cases the fitting of the electrodes must have started the waking process, as is indicated by the relatively rapid heart rates which were illustrated. In later work (Dawe and Morrison, 1955; Lyman and O'Brien, 1960), when chronically implanted electrodes were used, A-V dissociation has not been observed in hedgehogs or ground squirrels and it may be that it, like the notched R wave, is more typical of early arousal than of deep hibernation.

The effects of drugs, introduced via the aortic cannula, give some further insight into the control of circulation during hibernation. As far as we are aware, this approach to the problem has been undertaken only on the thirteen-lined ground squirrel. A few parallel experiments have been performed on the golden mantled ground squirrel with identical results, but generalizations, even about the genus Citellus, are perhaps premature (Lyman and O'Brien, 1960; Lyman and O'Brien, In manuscript).

The hearts of mammals in hibernation are extremely sensitive to stimuli, either from peripheral sources or from drugs introduced into the blood stream, and the response is almost invariably an increase in heart rate. It is not surprising, therefore, that the sympathomimetic norepinephrine causes a rapid increase in heart rate and pulse pressure and a rise in blood pressure. If infusion is continued, the animal starts the arousal process. The rise in blood pressure is so rapid that the slope of the diastolic run-off cannot be compared to that recorded before introduction of the drug, so that an estimate of the change in peripheral resistance is not possible.

The sympatholytic agent β -TM10 ([2- (2, 6-Dimethylphenoxy) propyl]-trimethylammonium chloride hydrate) is known to have an initial cardio-acceleratory action in other mammals, and this manifests itself in the

hibernating animal as well, accompanied by a concurrent rise in blood pressure. If the cardioacceleration is not so prolonged that it starts the process of arousal, the heart rate slows again to about the original rate. The drug causes a lower pulse and blood pressure and a more rapid diastolic run-off, which indicates that sympathetic tone has been maintaining vascular constriction during normal hibernation. The importance of constriction and relatively high peripheral resistance in hibernation is emphasized by the fact that the pharmacologically sympathectomized hibernating animal must be rewarmed artificially a few hours after the drug has taken effect or it will die.

The effects of the parasympathomimetic agents, acetylcholine and methacholine, are surprising, for both invariably cause cardioacceleration when infused into the circulation of a hibernating ground squirrel. This acceleration may appear in several forms. When low doses of acetylcholine are given, there may be a drop in blood pressure followed by an increase in heart rate which causes the blood pressure to at least equal its original value. This increase in heart rate may well be regarded as compensatory (Lyman and O'Brien, 1960). At other times, however, the heart rate increases at once, often within the next beat after infusion of the drug, with no time for an observable change in blood pressure or peripheral resistance. Quite rarely the blood pressure, peripheral resistance and heart rate remain unchanged for as long as several minutes, then the heart rate increases and the blood pressure rises.

It has not been possible to slow the normal heart rate of deeply hibernating ground squirrels either with massive infused doses of acetyl- or methacholine, or with high voltage stimulation of the vagus nerve via chronically implanted electrodes. This is not because the heart is beating at the lowest possible rate for that temperature. If the heart is accelerated by infusion of a parasympathomimetic or by electric shock, it cannot be immediately slowed either by another infusion of the drug or by vagal stimulation. After the heart has been beating at the new accelerated rate for several minutes, however, infusion of parasympathomimetics or vagal stimulation will cause a prolonged asystole, even though the heart temperature has remained unchanged. Thus it seems that some relatively slow, unknown change in the internal milieu has changed the threshold of the cardiac tissue.

There is usually 9 to 15 seconds delay after the parasympathomimetic has been introduced before the slowing of the heart occurs. This interval represents the circulation time from the aortic cannula to the heart, and is reduced if the drug is introduced via a cannula in the jugular vein. The lapse of time after injection before slowing occurs contrasts sharply with the rapid onset of cardioacceleration which may take place when acetylcholine is infused into the undisturbed hibernating animal. This strongly

suggests that the cardioacceleration is neurogenic and not due to some peculiar direct effect of the drug on the heart itself, such as release of endogenous cardiac catechol amines.

Ganglionic or sympathetic blockade gives some insight into the mechanism of cardioacceleration by parasympathomimetics during hibernation. When either hexamethonium or the sympatholytic agent β -TM10 is introduced into the aorta of the hibernating ground squirrel, an initial cardioacceleration is followed by a return of the heart to approximately its previous rate, a decline in peripheral resistance, and a drop in blood pressure. If acetylcholine is then introduced, the heart rate remains unchanged or is slowed and there is a complete collapse of blood pressure, so that injections of norepinephrine are often necessary to save the animal from death through circulatory failure. Pharmacologically sympathectomized animals or animals with ganglionic blockade are also incapable of normal arousal and show no cardioacceleration when mechanically stimulated, though this is the invariable effect in the untreated hibernating animal. The effects of treatment with hexamethonium or β -TM10 strongly suggest that acetylcholine and methacholine bring about their result via the cardioaccelerator fibers of the sympathetic nervous system. Since methacholine has no ganglionic stimulating action in the doses used, the effect cannot be due to the nicotinic effect of the drugs on the sympathetic ganglia.

The effect of methacholine on hibernating animals differs from acetylcholine in that the cardioacceleration caused by the former is always delayed more than 30 seconds after injection, while cardioacceleration by acetylcholine can be almost immediate. This is paralleled by their nicotinic effects on the muscle of nembutalized ground squirrels at a body temperature of 37° C, for methacholine causes a respiratory gasp some time after infusion, while acetylcholine causes a leg twitch almost immediately. In the hibernating animal infusion of acetylcholine causes an immediate attenuated burst of muscle action potentials followed at once by cardioacceleration (Figure 7a), while infusion of methacholine causes a long delayed series of muscle action potentials with a slight respiratory movement. This is followed by cardioacceleration. Thus, the temporal relationships in the response to the two drugs indicate that the nicotinic effect on skeletal muscle is closely associated with cardioacceleration.

Bursts of muscle action potentials occur when the hibernating hamster (Lyman and Chatfield, 1950) or ground squirrel (Lyman and O'Brien, In manuscript) is touched or physically stimulated. These continue for several seconds after the stimulus has been removed and cardioacceleration follows immediately (Figure 7b). Again the relationship of muscular activity — which usually can only be detected electrically — and cardioacceleration is too obvious to ignore and suggests that some reflex is set in motion either by

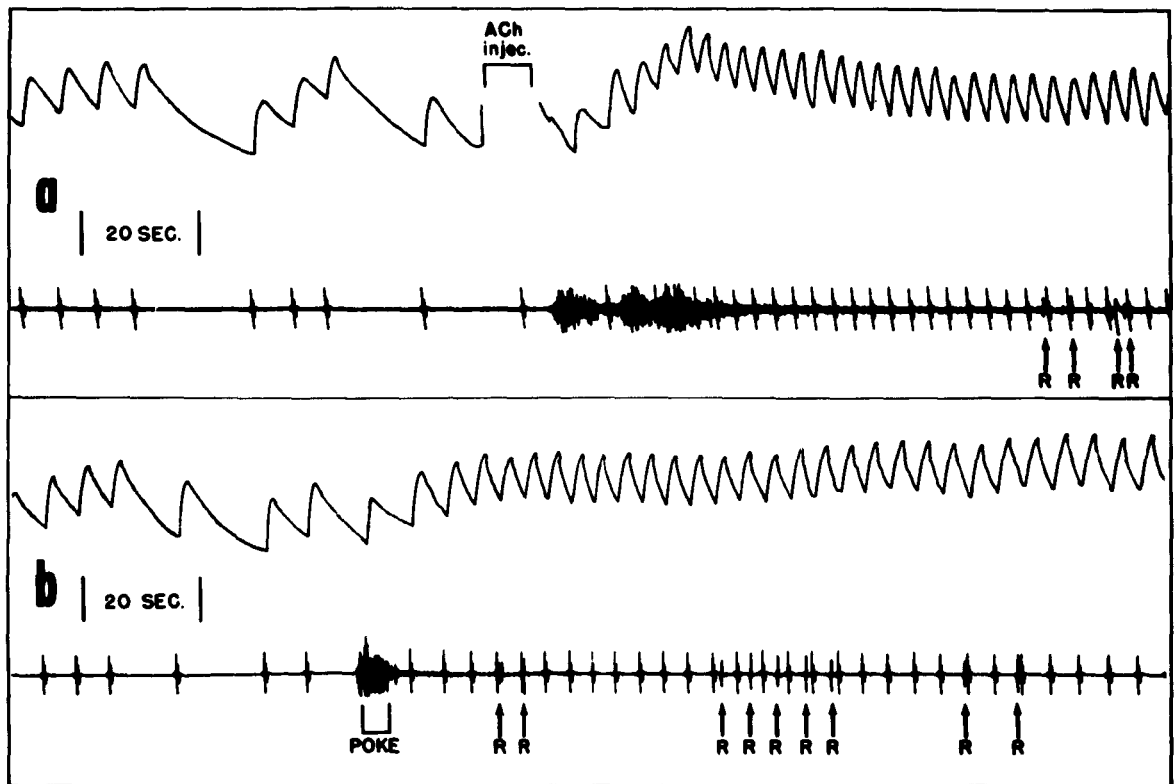


FIGURE 7a

Cardioacceleration in hibernation immediately after infusion via aortic cannula of 0.21 mg/kg acetylcholine. Heart temperature = 9° C. Note long-lasting burst of muscle action potentials on EKG. Continuous slow infusion of heparin saline invalidates absolute figures for blood pressure. R = respiration.

FIGURE 7b

Cardioacceleration in hibernation immediately after stimulating animal by poking with a stiff wire. Note similarity with Figure 7a. Heart temperature = 8.6° C. Animal on continuous slow infusion.

mechanical stimulation or by the nicotinic effect of parasympathomimetics which results in an increase of the heart rate. The nature of this reflex is not known but it seems to depend on the actual induced activity of the muscles, for the ground squirrel curarized in deep hibernation shows no cardioacceleration when treated with acetylcholine. The prolonged burst of muscle action potentials after stimulation seems to be typical of hibernation and it may be that this is the trigger which fires the arousal process into action.

The parasympatholytic agents atropine and banthine shed some further light on the control of the circulation in the hibernating animal. As the ground squirrel approaches deep hibernation, the heart rate may be relatively slow ($8 \pm$ beats/minute) while the temperature next to the heart is still five or more degrees above the environmental temperature of 5° C. Introduction of either of these drugs at this time causes a doubling or tripling of the heart rate with an abolishment of all skipped beats over long periods of time. The animal's body temperature continues to approach the environmental temperature but with a much faster heart rate. The effect of these drugs on the deeply hibernating animal with a heart rate under ten a minute is quantitatively different. In this case infusion usually causes a more even spacing of the beats, but the heart rate sometimes remains unchanged and at other times increases only one or two beats a minute. Thus the parasympathetic influence seems to fade as the animal levels off in deep hibernation. It is not known whether this diminution of parasympathetic influence is due solely to the aforementioned increasing threshold of the hibernating heart, or whether lessening of vagal tone is also involved.

The alkaloid veratramine slows the mammalian heart and antagonizes the cardioaccelerator action of epinephrine apparently by direct action on the pacemaker (Kramer, 1949). When infused into the hibernating animal it produces no change in the heart rate. This would seem to indicate that the heart in hibernation is beating at its slowest rate for the particular temperature and is not being influenced by the sympathetic system, but does not explain how a heart rate can be three beats/minute one day and eight beats the next with no detectable change in heart temperature.

What, then, is the general picture of the circulation of the animal in deep hibernation? It seems clear that vascular tone is maintained during hibernation, which results in a relatively high peripheral resistance. Vasodilators such as acetylcholine and methacholine will reduce this resistance. The peripheral resistance can be reduced by the adrenergic blocking agent Benodaine (Barcroft, 1934) and it can be practically abolished by the sympatholytic β -TM10 and the ganglionic blocking agent hexamethonium. It thus seems clear that the vascular tone is maintained in large part by sympathetic vasoconstrictor action. The vascular tone is not, however, comparable to the highly differential vasoconstriction which is described below in the waking hibernator.

Temperatures in all parts of the body are virtually the same during hibernation, and uptake of rubidium⁸⁶ by the tissues is proportionately about the same as it is in the active animal at a body temperature of 37° C (Bullard and Funkhauser, 1962). Thus, blood flow must be relatively unimpeded throughout the whole system. In all hibernating animals in which pigment does not obscure it, the feet are reported to be pink. This color must be due in part to the bright crimson of the well oxygenated blood, but it must also mean that complete vasoconstriction is not imposed at this time.

The concept that the degree of vasoconstriction in various parts of the body varies from time to time fits well with the observed facts. The variable blood pressure reported by us and the variations in skin temperature seen in the California ground squirrel (Strumwasser, 1960) suggest some such alternation of blood flow, possibly in response to accumulation of metabolites. The changes in heart rate over long periods of hibernation could be intimately connected with the changes in the vascular bed, though we have not observed a clear correlation of heart rate and blood pressure. A hibernating ground squirrel, with the same body temperature and heart rate, may show wide differences in its threshold to an infused drug from day to day. This also suggests variations in the vascular bed which permit the drug to reach different areas at different times.

The actual rate of the heart in hibernation cannot be completely explained by our knowledge to date. It is clear that the heart is slowed by vagal action as the animal approaches the deeply hibernating state, but parasympathetic influence seems to be minimal once deep hibernation is reached. Since atropine often changes the patterns of beats in the hibernating animal, it may well be that vagal influence is always present but can make little impression until the heart rate becomes "too high" for hibernation at that temperature. However, increase in rate does not immediately increase the effect of artificially imposed parasympathetic influence, and some more subtle changes must take place before the threshold of the heart is lowered.

Slowing the inherent rhythmicity of the heart in hibernation does not change the rate, so it is reasonable to assume that changes in inherent rhythmicity are not the cause for changes in rate during this period. On the other hand, the slow heart of the animal in hibernation is always ready to increase its rate and the change can take place in as little as two to three seconds after stimulation. Since atropinization does not necessarily increase the heart rate at all, the indications are that sympathetic influence on the heart is also at a low ebb, for parasympathetic blockade should reveal any sympathetic influence that might have been suppressed. If this is so, then the heart rate in hibernation is basically dependent on the activity of the sympathetic system.

The cardioaccelerator fibers are strongly implicated in the rapid speeding of the hibernating heart for any reason from the evidence cited above with norepinephrine, acetyl- and methacholine, hexamethonium and β -TM10. On the other hand, cardioacceleration which appears some time after the application of the stimulus, or which is of long duration with the animal remaining in hibernation, might be caused by the release of epinephrine from the adrenal medulla. By the same token the slow changes in heart rate which occur during a prolonged period of hibernation could be dependent on changes in circulating epinephrine from the same source. If this is the case, it is peculiar that veratramine does not slow the heart of the animal in deep hibernation.

The sympathetic and parasympathetic systems thus play an important balancing homeostatic role during the entrance into hibernation just as they do in normal active mammals, hibernators or not. It is perhaps to be expected, but it is still remarkable, that the autonomic system continues to function throughout the time that the body temperature is dropping more than 30° C. Once deep hibernation is reached, it is the sympathetic system which maintains vascular tone and probably influences the day to day changes in heart rate. Furthermore, it remains on guard to cause rapid cardioacceleration (and vasoconstriction) if the animal is disturbed. Its importance is emphasized by the fact that pharmacological damage to the system blocks normal arousal and results in death of the animal while still in hibernation. In contrast, blocking of the parasympathetic system has little effect on deep hibernation or the arousal from it.

Arousal

The hibernating mammal is always poised, like a resting athlete, to suddenly change to a more active state. Because the first changes in arousal cannot be observed grossly, the simile may seem overdrawn; yet an arousing hibernator can quintuple its heart rate in less than a minute, which compares favorably with the athlete. Once arousal is well under way it almost always is carried to completion, though on rare occasions with some species the animal may return to hibernation without warming to 37° C.

It is characteristic of all hibernators that arousals occur periodically during the hibernating season, though the bouts of hibernation vary from species to species and also with the time of year. The golden hamster may average three days in hibernation between arousal, while the average for the thirteen-lined ground squirrel is closer to six or seven days (Pengelley and Fisher, 1961), and bats remain torpid for more than a month. Kayser (1952) has shown that bouts of hibernation are longer during the middle of the hibernating season and this has been confirmed by Pengelley and Fisher (1961). The latter authors have also shown that the adult males of Citellus lateralis tend to hibernate for shorter periods than the females or juveniles.

Natural arousals from hibernation are generally as unpredictable as the entrance into hibernation and are therefore as difficult to study, but evoked arousals can be easily produced and this phase of the hibernating cycle is thus fairly well documented. The few observations on natural arousals do not differ from evoked arousals, and it is justifiable to assume that the same sequence of events occurs in either case. The cause of natural arousal is a mystery, though Strumwasser (1960) has shown that it occurs at the same time of day in the California ground squirrel which is starting the hibernating season, as if the stimulus was mediated by some sort of temperature independent biological clock. Many other hibernators demonstrate no particular diurnal pattern in arousal time, but arousals take place at fairly regular intervals (Pengelley and Fisher, 1961).

The stimulus necessary to cause an evoked arousal varies from species to species and even within the same individual at different time in the cycle. Sometimes a hibernating golden hamster will arouse if the fur of the back is lightly touched, while even the insertion of a rectal thermocouple may fail to cause complete arousal in a thirteen-lined ground squirrel. Animals hibernating at higher environmental temperatures are more sensitive than animals in which the body temperature approaches 0° C, and it is also true that the bouts of hibernation are shorter if the body temperature and environmental temperature are high.

When evoked arousal is initiated, the first observable changes are a burst of muscle action potentials and an increase in respiratory rate, followed by cardioacceleration. In thirteen-lined ground squirrels this is accompanied by a decrease in peripheral resistance so that the blood pressure does not necessarily rise at once with the increasing heart rate. As arousal continues, however, and the heart rate becomes more rapid, the blood pressure also rises (Figure 8). The heart rate may increase 100-fold as the animal arouses from hibernation, yet the electrocardiogram shows few anomalies. At the very start of arousal A-V block may occur (see "In Hibernation") but this is soon abolished as arousal progresses.

In spite of the immediate and important appearance of muscle action potentials once the animal is stimulated, shivering does not become grossly visible until arousal is well under way. However, at the peak of the waking process, when the heart temperature is approaching 37° C, shivering is violent, especially in the anterior portion of the animal. Oxygen consumption rises and reaches its maximum value at about the time the temperature of the anterior reaches 37° C, after which it drops to a lower value (Figure 9). The whole process of arousal is a stupendous physiological effort in which the maximum oxygen consumption is greater than in an animal under violent conditions of exercise (Benedict and Lee, 1938). Indeed Adolph and Richmond (1955) have calculated that the metabolic expenditure of one arousal is equal

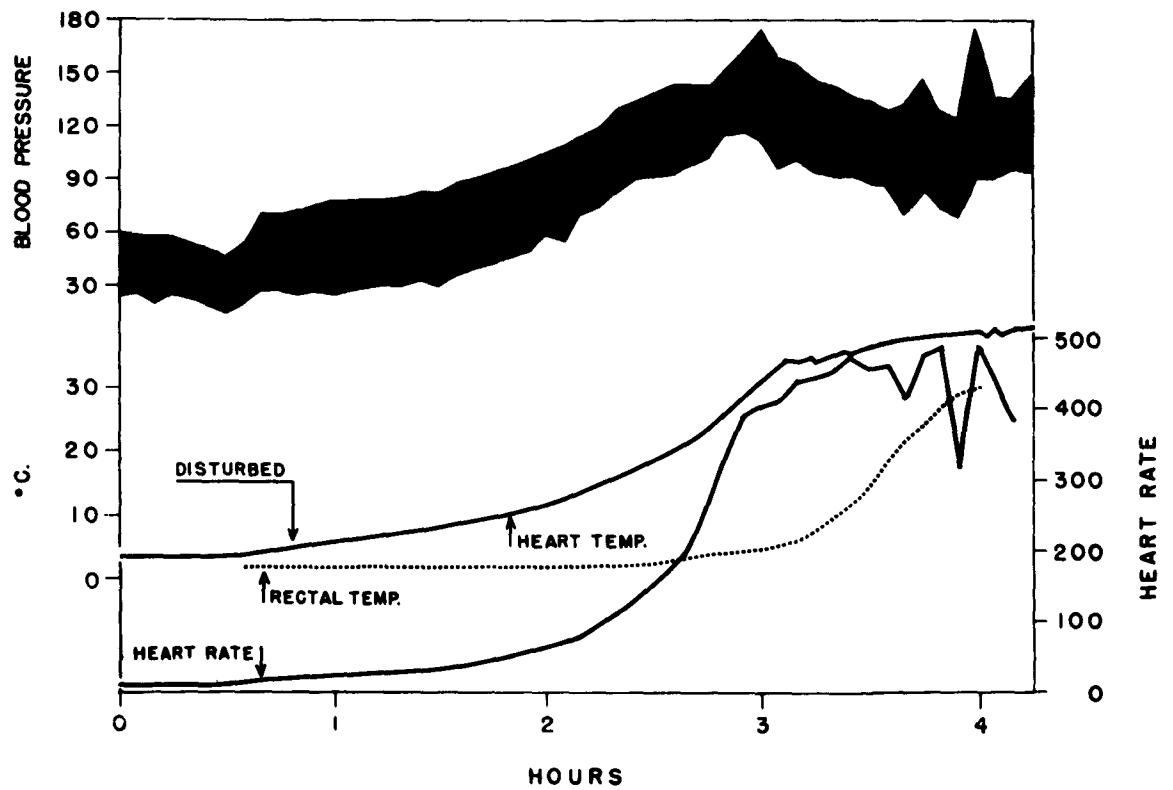


FIGURE 8

Ground squirrel arousing. Blood pressure recorded as in Figure 1. Note difference in heart and rectal temperature.

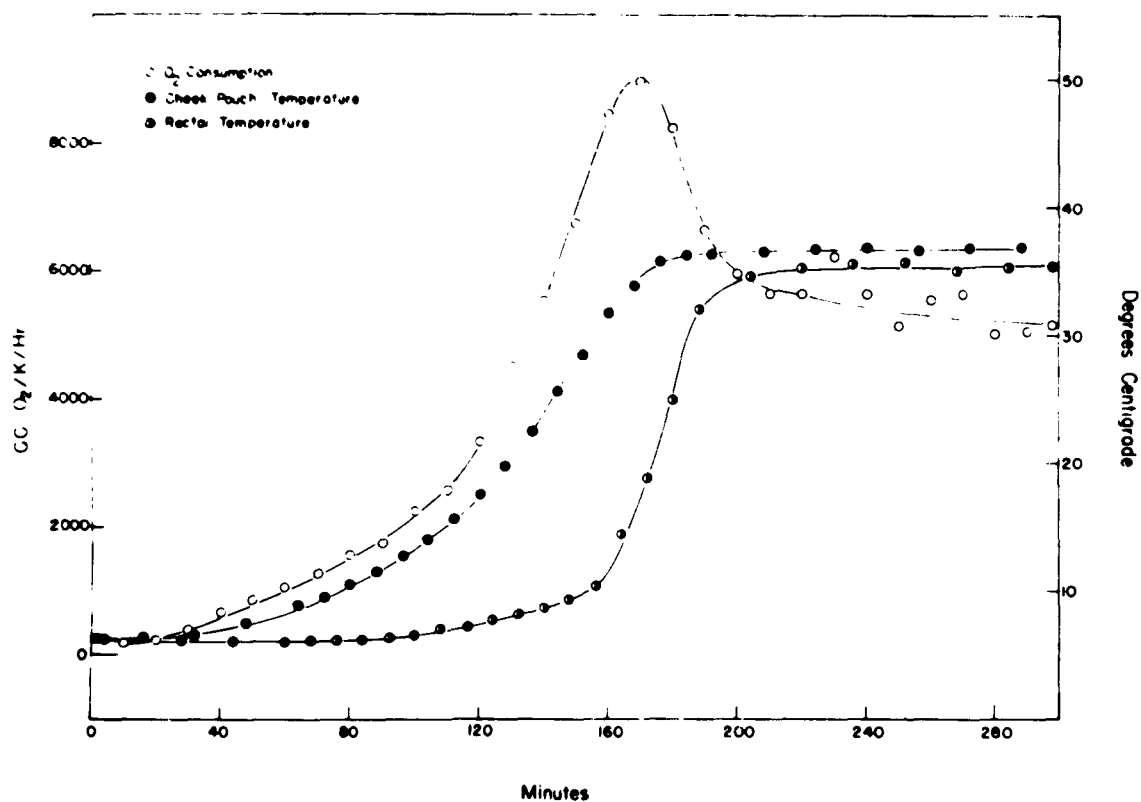


FIGURE 9

Hamster waking from hibernation. Note overshoot of oxygen consumption and difference in cheek pouch (thoracic) and rectal temperature.

to ten days of deep hibernation. During this time, piloerection increases the insulation, but the circulatory adjustments are even more important in heart conservation.

At the start of arousal the temperature in all parts of the body is nearly the same, and Bullard and Funkhauser (1962) have shown, using tracer techniques, that blood flow is distributed much as it is in the awake animal, though, of course, the flow is much slower. After the heart rate and respiratory rate have increased, the anterior of the animal begins to warm, but the posterior remains cold. The gradient between thoracic and deep abdominal temperature increases as warming continues, so that the difference may be as much as 20°C at about the time the oxygen consumption reaches its maximal value (Figure 8). All hibernators which have been studied, including bats, demonstrate this same method of rewarming, though the aestivating Mohave ground squirrel may warm its whole body at once when arousing from relatively high body temperatures (Bartholomew and Hudson, 1960). The economy of differential rewarming greatly shortens the time involved in returning to the warmblooded state, for the amount of heat lost from the body surface during the first two-thirds of the warming process is greatly reduced. Once the anterior portion, containing the large muscle masses as well as the important organs involved in the warming process, is well heated, vasodilation occurs and the posterior warms rapidly.

As early as the 1890's Marès (1892) and Dubois (1896) had reported that dyes injected into the circulation of the hibernating animal first appeared in the anterior portion of the body. It is not clear whether they realized that this situation was typical of arousal rather than deep hibernation, but there can be no doubt that the process of injecting either by vein or by artery must have started the arousal process. Exposure of the viscera showed that the dyes appeared in the arteries there, but that little reached the portal system. These observations were confirmed more than 50 years later in intact arousing hamsters using cardiac injections of radiopaque fluid and X ray (Lyman and Chatfield, 1950). In all cases flow of the material to the posterior was greatly impeded when compared to anaesthetized controls with body temperature of 37°C . This, plus a blanching of the feet, constitutes good evidence that a peripheral and posterior vasoconstriction occurs early in arousal.

Recently quantitative evidence of blood flow to various organs during arousal has been presented by Johansen (1961) using the Arctic ground squirrel and by Bullard and Funkhauser (1962) with the thirteen-lined ground squirrel. The technique employed with both species was the measurement of the accumulation of rubidium ⁸⁶ in various tissues and organs after injection into the blood stream. Although Johansen measured blood flow later in the arousal process, the general conclusions on the two species are very similar. The accumulation of the isotope was proportionately higher in tissues and organs in the anterior part of the body and remained virtually

unchanged in the abdominal viscera and the posterior in general. By measuring mean stroke volume (0.15 ml) in the thirteen-lined ground squirrel, Bullard and Funkhauser were able to calculate cardiac output and, from the proportional distribution of rubidium ⁸⁶, the blood flow per gram in the various tissues. As might be expected, the flow to the heart muscle, diaphragm, thorax and forelimbs increased markedly during arousal. A surprising result reported from both laboratories was the great increase of blood flow to the brown fat, both interscapular and axillary. This strongly suggests that this so-called "hibernating gland" may be a source of quick energy during the arousal process and should more properly be called, as Bullard has suggested, the "de-hibernating" gland.

Blood flow to the posterior and periphery did not decrease as heart rate and anterior temperature increased, but rather remained about the same as in deeply hibernating animals. Since blood pressure is increasing during this time, it is reasonable to assume that vasoconstriction of the posterior is also increasing and thus maintaining flow at nearly the same level. In spite of this differential vasoconstriction, 30% to 40% of the blood leaves the thoracic region at a heart rate of 100 beats per minute, and this is certainly sufficient for maintenance of the less active areas. Interestingly enough, the hypothermed rat shows little of the hibernator's ability to confine the major flow of blood to the anterior, though it does have some vasoconstriction in feet and tail (Bullard and Funkhauser, 1962).

These detailed studies in blood flow provide additional information on the sources of heat in the arousing hibernator. Dubois (1896), using ligation techniques, had concluded that the liver was the principal source of heat during arousal. However, it was shown many years later (Lyman and Chatfield, 1950) that the eviscerated hamster, with blood flow to the liver ligated, would arouse from hibernation as rapidly as a normal animal, with the conclusion that the failure of arousal in Dubois' marmots was due to pooling of blood in the splanchnic bed. The rubidium ⁸⁶ experiments show that blood flow to the liver is not augmented as much as it is to muscles, diaphragm and heart during arousal, which is further evidence that the liver is not a major source of heat during this time. On the other hand, liver glycogen is greatly depleted during arousal in the hamster, though blood glucose never sinks below normal. In the eviscerated animal with the liver ligated, arousal can be completed, but blood glucose drops to hypoglycemic levels (Lyman and Leduc, 1953). Thus, the liver must supply a sizable portion of the readily mobilized energy necessary for arousal, and the moderate increase in blood flow which was found in this organ is to be expected.

If the hibernating hamster is curarized after evisceration and given artificial respiration, it is capable only of a very slow, partial warming from the hibernating state (Lyman and Chatfield, 1950), thus emphasizing

the importance of muscular activity as a source of heat. The great increase in blood flow to the thoracic muscles and diaphragm in the ground squirrel is additional evidence of the importance of these tissues in the warming of hibernators during arousal.

It is surprising that the curarized eviscerated animal can warm at all, and we have suggested that the rapidly beating heart may supply some of the heat. The heart is moving blood which is viscous from the cold and the heart muscle must itself be viscous. Furthermore, the rising blood pressure must increase the work of the heart. These factors may make the heart an inefficient pump but must increase its role as a heat generator. Again, the high blood flow found in the coronary muscles confirms the importance of the heart in the warming process. It was realized, however, that heat from the heart was not sufficient to warm the eviscerated curarized animal (Lyman and Chatfield, 1950) and we postulated an energy-wasting uncoupling of phosphorylation to supply the needed warming. The high blood flow to brown fat suggests an alternate source of heat. Recently it has been shown (Ball and Jungas, 1961) that white adipose tissue, under hormonal influence, is broken down to fatty acid and glycerol and that the fatty acid is then re-esterified by way of glycerol phosphate furnished by glucose. This apparently purposeless process, if carried on in the metabolically active brown fat, could contribute significantly as a source of heat to the arousing hibernator.

Vascular changes are reflected in changes in blood pressure as arousal progresses. After the first decrease in peripheral resistance and increase in heart rate in the thirteen-lined ground squirrel, the heart rate continues to increase and the blood pressure rises steadily. The rise in blood pressure is initially very rapid in the golden hamster (Chatfield and Lyman, 1950) but is slower in the ground squirrel (Figure 8). Blood pressure remains high during the remainder of arousal in the latter animal, but usually declines somewhat as the deep abdominal temperature starts to rise (Lyman and O'Brien, 1960).

It seems probable that the initial decrease in peripheral resistance which appears at the start of arousal is caused by a vasodilation in the anterior portion of the mildly vasoconstricted deeply hibernating animal. If one of the requisites of a fast arousal is a rapidly beating heart, then a decrease in peripheral resistance could shorten the ejection time and help speed the heart rate. After the first few minutes, however, the blood pressure starts to rise, and the evidence given above would indicate that vasoconstriction of the posterior and the periphery becomes increasingly severe. The decline in blood pressure which often occurs as the posterior starts to warm rapidly can be explained by the sudden increase in the size of the vascular bed as posterior and peripheral vasodilation finally takes place.

That the posterior vasoconstriction is mediated by the sympathetic nervous system can be demonstrated by pharmacologically sympathectomizing the hibernating thirteen-lined ground squirrel with β -TM10. If such an animal is then stimulated to arouse, the deep abdominal temperature rises almost as rapidly as the temperature near the heart (Figure 10) (Lyman and O'Brien, In manuscript). The importance of vasoconstriction is re-emphasized in such a preparation, for the time taken to warm the whole body is much longer than in the normal animal. Similar attenuations of the warming period have been observed in golden hamsters which warmed the whole body at once after radical surgical procedures (Lyman and Chatfield, 1950).

The differential vasoconstriction of arousal might be accomplished if the posterior and periphery had a lower threshold to circulating norepinephrine than the anterior. We have postulated that arousal is mediated by a spontaneous discharge of the sympathetico-adrenal system (Chatfield and Lyman, 1950). If this is the case, blood catechol amines should be high and could cause local vasoconstriction. That this does not occur can be demonstrated by quickly infusing large doses of norepinephrine into the blood stream of the arousing hibernator which has been pharmacologically sympathectomized with β -TM10. (β -TM10 does not inhibit the effect of norepinephrine at the effector cell.) Such infusions do not produce a retardation of the rate of warming of the posterior which would indicate vasoconstriction in that area. This negative evidence implies that the differential vasoconstriction of arousal is produced by the discrete action of sympathetic vasoconstrictor fibers rather than a difference in threshold to norepinephrine from anterior to posterior.

The method of control of the circulation during arousal, and the neuro-humors involved, can be demonstrated by other pharmacological experiments. If the ground squirrel arousing from hibernation with its abdominal temperature at a low level is quickly infused with acetylcholine via the aortic cannula, the temperature near the heart stops its rapid rise and the deep abdominal temperature, which had remained steady, rises steeply for several minutes (Figure 11). In this case acetylcholine has caused vasodilation of the posterior, so that the warm blood of the anterior heats the posterior and returns to chill the anterior and slow its rate of warming. On the other hand, if norepinephrine is infused rapidly into the ground squirrel when the anterior temperature has reached 37° C and the posterior is warming rapidly, then the warming of the posterior is slowed for several minutes (Figure 12). Continuous infusion of norepinephrine will not maintain the vasoconstriction, but if the drug is introduced at even intervals the temperature of the posterior may rise in a stepwise manner as norepinephrine briefly reimposes vasoconstriction.

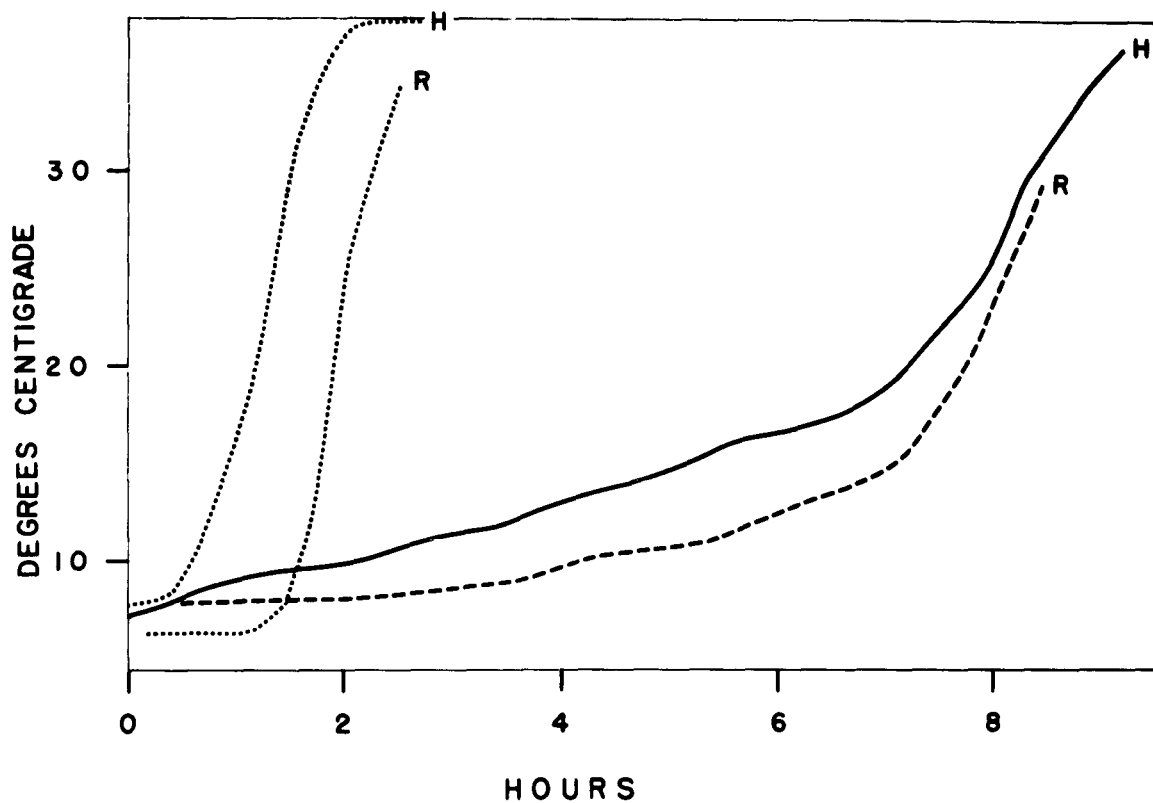


FIGURE 10

Ground squirrel arousing from hibernation after treatment with sympatholytic β -TM10 (_____ heart temperature; ----- rectal temperature), compared with untreated arousal (.....). Note attenuation of arousal and lack of vasoconstriction of posterior in animal that received β -TM10.

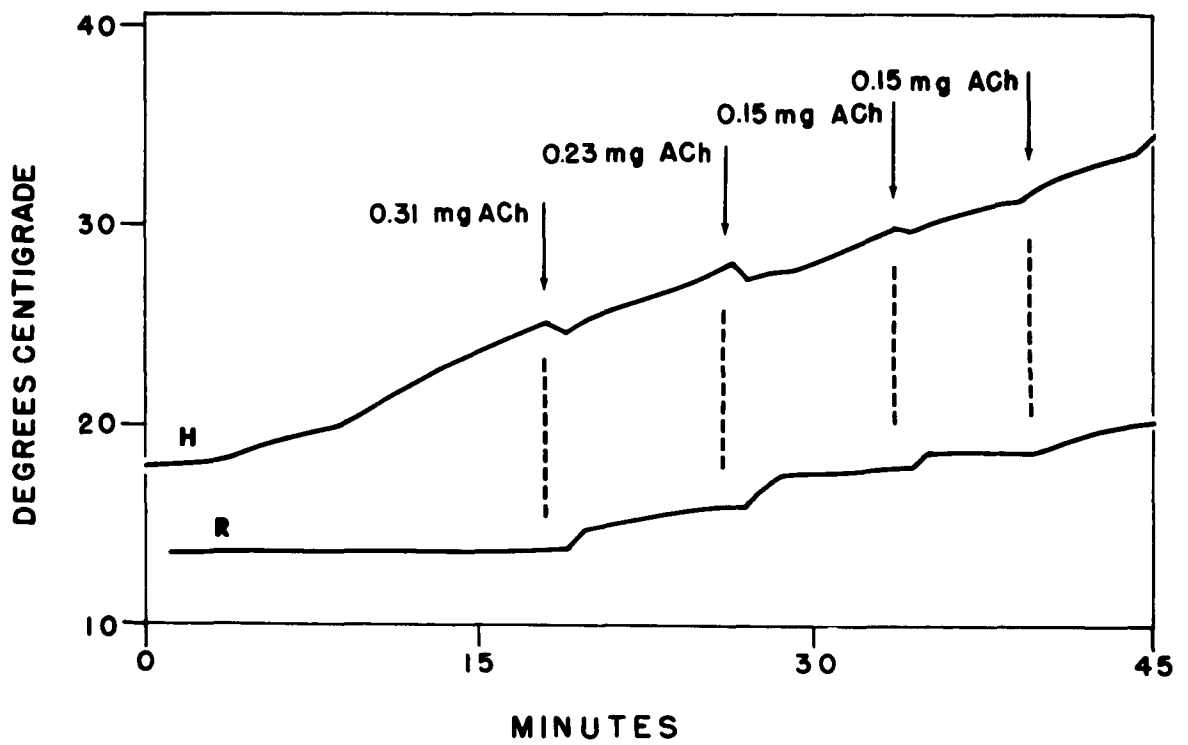


FIGURE 11

Effect of acute successive infusions of acetylcholine via aortic cannula in ground squirrel arousing from hibernation. Vasodilation in posterior causes transient rise in rectal temperature and drop in heart temperature.

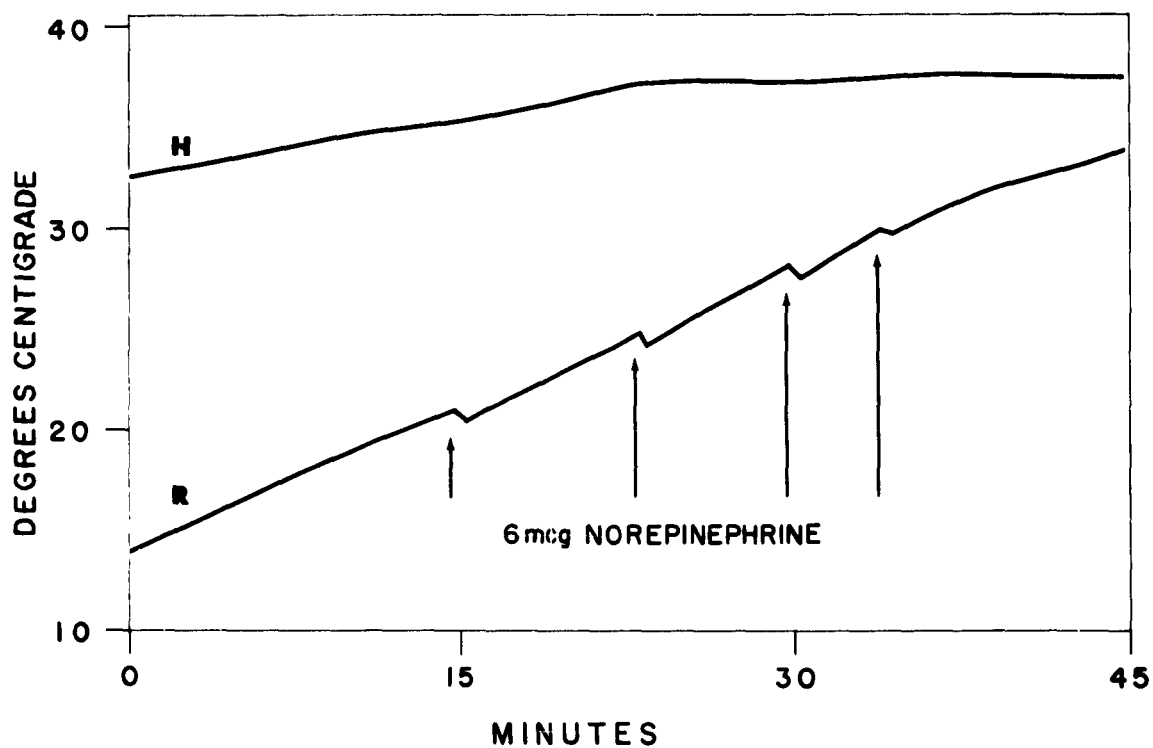


FIGURE 12

Effect of rapid infusion of norepinephrine in arousing ground squirrel when heart temperature has approached 37°C and rectal temperature is rising rapidly. Drug reimposes vasoconstriction briefly, stopping rise of rectal temperature for a short period.

Thus, arousal from hibernation appears as a precisely organized series of events in which the circulation plays an essential role, especially in the control of the distribution of heat. While the entrance into hibernation seems to be purposefully designed to gently shift the animal from the steady warm-blooded state to the steady state of hibernation with the least physiological trauma, the arousal process is a violent one in which the maximum effort is put forth to force the animal from hibernation in the least possible time. The muted entrance into hibernation is metabolically cheap and there is no evidence that any animal hastens the process by expensive devices such as evaporative cooling. On the other hand, arousal is metabolically an extremely costly process, and the whole animal seems to be organized to get it over as quickly as possible. In entering into hibernation and in the hibernating state the chief function of the circulation is the carrying of nutrients and the removal of metabolites. In arousal, the distribution of heat by the circulation is of almost equal importance.

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